PCR (RT-qPCR). The level of membrane CD137 was measured by Flow Cytometry (FCM). The levels of NF-κB p65,phospo-NF-κB p65(p-p65) and NFATc1 of the VSMCs were determined by Western blot. TRAF6 was blocked by small interfering RNA(siRNA). MTT was used to observe the cell proliferation. The cell supernatant of IL-2 and IL-6 were tested by ELISA.

RESULTS The level of CD137 was induced by TNF-α in smooth muscle cells after stimulating 24h(40.00±2.83 vs 1.00±0.00,p<0.05). The cells proliferated when treated with the agonist-CD137 mAb. The mRNA level of NFATc1 was increased after stimulated by agonist-CD137 mAb(2.07±0.09 vs 1.00±0.00, p<0.05), the protein of NFATc1 was also increased. Simultaneously, the mRNA level of TRAF6 was also increased(1.39±0.16 vs 1.00±0.00,p<0.05) and p-p65 protein was also increased(p<0.05). When TRAF6 was blocked by TRAF6 siRNA,the expression of p-p65 and NFATc1 was decreased after stimulated by agonist-CD137 mAb compared with the stimulated group(1.15±0.07 vs 2.07±0.09, p < 0.05). Pretreated the VSMCs with PDTC(30μmol/L) after 30min,the expression of p-p65 was inhibited in cytoplasm and nucleus,we stimulated the cells by agonist-CD137mAb at this time,the mRNA level of NFATc1 was also suppressed(1.15±0.07 vs 2.07±0.09, p < 0.05) and the NFATc1 protein was inhibited(p < 0.05). The cell supernatant of IL-2 and IL-6 went up by agonist-CD137 mAb(0.91±0.1 vs 1.29±0.17,p<0.05;0.51±0.04 vs 0.61±0.07,p<0.05), and decreased when NFATc1 was silenced(1.29±0.23 vs 1.00±0.00, p<0.05;0.61±0.07 vs 0.50±0.03,p<0.05).

CONCLUSIONS These results demonstrates that CD137 can be induced by TNF-α in VSMCs, and the CD137 may signaling affect the expression of NFATc1 in mice VSMCs through TRAF6/NF-κB pathway.

GW26-e0806

Intracoronary Cardiosphere-Derived Cells for Heart Regeneration After Myocardial Infarction

Jie Qin, Yuefei Guo, Xiuzhen Chen, Xuelian Liu
Department of Radiology, the Third Affiliated Hospital of Sun Yat-sen University

OBJECTIVES We aimed to assess safety of therapy with cardiosphere-derived cells (CDCs) in patients with left ventricular dysfunction after myocardial infarction.

METHODS An independent data coordinating center randomly allocated patients in a 2:1 ratio to receive CDCs or standard care. For patients assigned to receive CDCs, autologous cells grown from endomyocardial biopsy specimens were infused into the infarct-related artery 1·5-3 months after myocardial infarction. The primary endpoint was proportion of patients at 6 months who died due to ventricular tachycardia, ventricular fibrillation, or sudden unexpected death, or had myocardial infarction after cell infusion, new cardiac tumor formation on MRI, or a major adverse cardiac event. We also assessed preliminary efficacy endpoints on MRI by 6 months.

RESULTS Between May 1, 2009, and Dec 30, 2014, we randomly allocated 31 eligible participants of whom 25 were included in a per-protocol analysis. Mean baseline left ventricular ejection fraction (LVEF) was 39% (SD 12) and scar occupied 24% (10) of left ventricular mass. Biopsy samples yielded prescribed cell doses within 36 days (SD 6). No complications were reported within 24 h of CDC infusion. By 6 months, no patients had died, developed cardiac tumors, or MACE in either group. Four patients (24%) in the CDC group had serious adverse events compared with one control (13%; p=1.00). Compared with controls at 6 months, MRI analysis of patients treated with CDCs showed reductions in scar mass (p=0.001), increases in viable heart mass (p=0.01) and regional contractility (p=0.02), and regional systolic wall thickening (p=0.015). However, changes in end-diastolic volume, end-systolic volume, and LVEF did not differ between groups by 6 months.

CONCLUSIONS Intracoronary infusion of autologous CDCs after myocardial infarction is safe and effective, warranting the expansion of such therapy to phase 2 study.

GW26-e1045

ITRAQ-Based Quantitative Proteomic Analysis of Heart in a Rat Model of Exhaustive Training

Haiyan Liu, Xuebin Cao
No. 252 Hospital of PLA

OBJECTIVES To explore the presence of informative protein biomarkers in the rat cardiac between the health group and exhausting group, The purpose of this study is to understand the difference in protein expression patterns between health and after exhaustive swimming and to evaluate the protein contributions to exhaustive training.

METHODS 20 male SD rats (male, the weight is 200±20g, SPF) were randomly divided two groups, health group and exhausting group. The rats of exhausting group were swimming in the artificial pond, get the swimming method result in a model of Single Bout of Exhaustive Swimming in Rats. After exhaustive swimming the hearts were collected immediately. We mainly adopted advanced 8-plex ITRAQ coupled with 2D LC-MS/MS technology for proteomics.

RESULTS Analysis of proteomic data found that 122 proteins were identified with quantitative information between the two groups, of with the expression level of 69 proteins had significant differences at least, compared with the health group(ratio > 1.2 or < 0.7, and P < 0.05) ,of which 50 proteins were up-regulated, 19 proteins down-regulated.

CONCLUSIONS This study provided a global view of potential mechanisms and potential biomarkers of heart, and demonstrated that iTRAQ combined with 2D LC-MS/MS quantitative proteomics is a powerful tool for biomarker discovery.

GW26-e1069

Exendin-4, An Gulcagon-like Peptide 1 Analogue, Attenuates Cardiomyocyte Hypertrophy Via AMPK/mTOR Pathway

Yue Zhou, Xin He, Yiyi Huang, Yili Chen, Jiangui He
Department of Cardiology, The First Affliated Hospital of Sun Yat-sen University

OBJECTIVES Cardiac hypertrophy is the pathological basis of the development of various cardiovascular diseases and is a major independent risk factor of cardiovascular morbidity and mortality. Glucagon-like peptide 1 (GLP-1), an incretin peptide released from the intestine, exerts various cardioprotective actions and is proved to contribute in the regulation of cardiac functions. However, the role of Exendin-4, a stable GLP-1 analogue, in the cardiac hypertrophy remains unclear.

METHODS 1. Primary neonatal ventricular cardiomyocytes were cultured to establish the model of cardiomyocyte hypertrophy induced by phenylephrine (PE).

2. RNA isolation and quantitative real-time PCR (q-PCR) were performed to evaluate the transcriptional level of hypertrophic markers such as ANP BNP and beta-MHC.

3. Western Blotting was carried out to observe the change of the signaling pathway proteins levels.

4. The cardiomyocyte morphological change was manifested by Immunofluorescence staining and measurement of the cell surface area.

RESULTS Our study demonstrated that exendin-4 attenuated cardiac hypertrophy induced by phenylephrine (PE), manifested by decreased hypertrophic markers such as ANP, BNP, beta-MHC and cell surface. Phosphorylated extracellular signal regulated protein kinase (phospho-ERK1/2) and phosphor-p38 mitogen-activated protein kinase (MAPK) protein levels didn't change in the team treated by exendin-4 and PE. In addition, we discovered that exendin (9-39), a GLP-1 receptor antagonist, can remove the anti-hypertrophic effect of exendin-4, evidently. Moreover, we showed that the anti-hypertrophic effect of exendin-4 was also significantly reversed by compound C, an AMPK inhibitor, and rapamycin, a selective blocker of mTOR.

CONCLUSIONS These results demonstrate that exendin-4 inhibit cardiac hypertrophy induced by PE via AMPK/mTOR pathway.

GW26-e1380

Vascular Adventitia Calcification and its Underlying Mechanism

Na Li, Wenli Cheng
China-Japan Friendship Hospital

OBJECTIVES Previous research on vascular calcification has mainly focused on the vascular intima and media. However, we show here that vascular calcification may also occur in the adventitia as well. The purpose of this work is to help elucidate the pathogenic mechanisms underlying vascular calcification.

METHODS Mice were fed high fat diets (HFD) for 48 weeks, observing calcified lesions. Also included in this study were human subjects aged 60 years and older that had died of coronary heart disease, heart failure or renal failure, observing calcified lesions. Explant culture of fibroblasts, the primary cell type comprising the adventitia, was

induced for calcification. Culture of smooth muscle cells (SMCs), which comprise only a small percentage of all cells in the adventitia, in calcifying medium resulted in calcification.

RESULTS Following 48 weeks, calcified lesions were observed in the aorta adventitia and coronary artery adventitia of ApoE-/-mice. Von Kossa staining showed calcification in the human aorta adventitia. Explant culture of fibroblasts, was successfully induced for calcification after incubation with TGF-β1 (20 ng/ml) + mineralization media for 4 days, and the phenotype conversion of vascular adventitial fibroblasts into myofibroblasts was identified. Culture of smooth muscle cells (SMCs), which comprise only a small percentage of all cells in the adventitia, in calcifying medium for 14 days resulted in significant calcification.

CONCLUSIONS Vascular calcification can occur in the adventitia. The conversion of fibroblasts into myofibroblasts may contribute to the calcification process. Although SMCs comprise only a small percentage of the entire adventitia cell population, they may also contribute.

GW26-e1388

Transplantation of EPCs Overexpressing S1PR3 Promotes Vascular Repair in the Early Phase After Vascular Injury

Hang Wang,[1] Keyin Cai,[1] Li Wang,[1] Kehong Zhao,[1] Ying Tu,[1] Xiao Wang,[1] Jiahuan Li,[1] Hao Huang[2]
[1]Cadre Ward Two, Wuhan General Hospital of Guangzhou Military Command, Wuhan 430070, China; [2]Clinic center, Shenzhen Hornetcorn Biotechnology Crop. Shenzhen 518400, China

OBJECTIVES Endothelial progenitor cells (EPCs) play important roles in the process of reendothelialization and prevent neointimal formation after vascular injury. Sphingosine-1-phosphate (S1P) can via the sphingosine-1-phosphate receptor 3 (S1PR3) induce proliferation,migration and angiogenesis of EPCs. This study aims to investigate effects of transplantation of EPCs overexpressing S1PR3 on reendothelialization and neointimal formation in response to vascular injury in mice.

METHODS Spleen-derived EPCs were cultured and expanded in endothelial basal medium. EPCs were infected with lentivirus vectors expressing mice S1PR3 (S1PR3- EPCs)or green fluorescent protein(GFP-EPCs). Three days after gene transfection, the mRNA level and protein level of S1PR3 were assayed. Mice were administered 200 μL saline alone or 200 μL saline containing fluorescently labeled EPCs (1×10^6) or fluorescently labeled EPCs with expressing S1PR3 (1×10^6) via tail vein injection directly after wire-mediated carotid artery injury and again after 24 h. 14 days after transplantation, cell tracking showed that transfused EPCs could homeing to the sites of endothelial injury.

RESULTS At day 7, the reendothelialized area in the S1PR3- EPCs transplanted arteries (56.32 ± 6.33%) was significantly larger than that in the GFP- EPCs transplanted arteries (42.47 ± 4.69%; $p<0.05$) and that in the saline control group (28.31 ± 7.32%, $p<0.01$). At day 14, the reendothelialized area in the S1PR3- EPCs transplanted arteries (72.28 ± 8.37%) was significantly larger than that in the GFP- EPCs transplanted arteries (50.21 ± 5.66%, $p<0,01$) and that in the saline control group (29.98 ± 7.58%, $p<0.01$). At day 14, a significant decrease ($P < 0.05$) in neointimal/media (NI/M)ratio was noted in the S1PR3- EPCs group (0.31 ± 0.02) as compared with that in the GFP- EPCs group (0.45 ± 0.10) and in the saline control group (0.77±0.21). Our results showed that EPCs overexpressing S1PR3 significantly accelerates reendothelialization and mitigates neointimal formation in the early phase after carotid artery injury in mice.

CONCLUSIONS Our data suggest that transplantation of EPCs overexpressing S1PR3 can have a combined effect of both amplifying the reendothelialization capacity of EPCs and inhibiting neointima formation so as to facilitate better inhibition of adverse remodeling after vascular injury. Therefore, gene modified EPCs may be applied in clinical progenitor cell therapy to improve vascular repair after vascular injury.

GW26-e1483

Aberrant Expression of Circular RNAs in Endothelial Dysfunction

Yuqiang Ji, Manli Cheng
First hospital of Xi'an

OBJECTIVES The aim of this study was to determine the expression profile of circular RNAs (circRNAs) in oxidized low-density lipoprotein (ox-LDL) treated human umbilical vein endothelial cells (HUVECs) compared with normal HUVECs through human circRNA microarray and predict their miRNA binding sites.

METHODS The global circRNAs expression profiles in ox-LDL treated HUVECs compared with normal HUVECs were measured by the Arraystar Human circRNA Array (8x15K, Arraystar). Agilent Feature Extraction software (version 11.0.1.1) was used to analyze acquired array images. The circRNA/microRNA interaction was predicted with Arraystar's home-made miRNA target prediction software.

RESULTS Compared with normal HUVECs, 24 circRNAs were different expression (fold change≥1.5, p-value cut-off is 0.05) in ox-LDL treated HUVECs. 15 circRNA were up-regulated and 9 circRNAs were down-regulated. The most up-regulated circRNA was circRNA-104137 and the most down-regulated circRNA was circRNA-100188. The miRNA binding sites of circRNA-104137 were miR-455-3p, miR-218-1-3p, miR-423-5p, miR-503-5p and miR-223-3p. The miRNA binding sites of circRNA-100188 were miR-637, miR-608, miR-328-5p, miR-877-3p and miR-185-3p.

CONCLUSIONS The aberrantly expression profile of circRNAs in ox-LDL treated HUVECs compared with normal HUVECs indicates the potential roles of circRNAs in endothelial dysfunction. This study may provide new insights into the mechanism and potential targets for the endothelial dysfunction.

GW26-e1596

Effects of atorvastatin on mRNA and protein expression of adropin in cultured human umbilical endothelial cells and rat artery smooth muscle cells

Liangping Zhao, Chengjia Zhang, Li Wang, Tao You, Weiting Xu, Jianchang Chen
Department of Cardiology, The Second Affiliated Hospital of Soochow University China

OBJECTIVES Adropin is a newly-identified secretary protein that participates in the regulation of energy homeostasis and insulin response. Growing published evidence presented the beneficial association of adropin with coronary artery disease. A laboratory test showed the beneficial effects of adropin on endothelial cells proliferation and migration. The endothelial cell dysfunction and proliferation of vascular smooth muscle cell form the core mechanism of atherosclerosis. Therefore, to increase the expression of adropin by some therapy may play a role in the prevention and treatment of atherosclerosis. In the present study, the effects of atorvastatin on mRNA and protein expression of adropin in cultured human umbilical vein endothelial cells (HUVEC) and rat artery smooth muscle cell (RASMC) were investigated.

METHODS HUVEC and RASMC were cultured in vitro with atorvastatin of 0.002, 0.02, 0.2, 2 and 20 umol/L for 6, 12 and 24 hours (h). The proliferation of HUVEC and RASMC were detected by MTT chromatometry. RT-PCR, and ELISA were performed to present the expression of adropin mRNA and adropin protein respectively.

RESULTS The proliferation of HUVEC was promoted after co-incubation with atorvastain from 0.002 to 20 umol/L concentration, and the peak was at 20umol/L (versus control, $P<0.05$) and 24 h (versus 6 h, $P<0.05$). Atorvastatin upregulated the expression of adropin mRNA and adropin protein dose-dependently and time-dependently in HUVEC with a peak at 20umol/L (versus control, $P<0.01$) and 24 h (versus 6 h, $P<0.01$). Adropin protein concentration in culture medium was positively correlated with the OD value indicating proliferation at 12 h ($P=0.001$) and 24 h ($P<0.001$) after co-incubation.

The proliferation of RASMC was decreased after co-incubation with atorvastain from 0.002 to 20umol/L concentration, and the valley was at 20umol/L (versus control, $P<0.05$) and 24 h (versus 6 h, $P<0.05$). Atorvastatin upregulated the expression of adropin mRNA and adropin protein in RASMC with a peak at 0.02 umol/L (versus control and 20 umol/L, $P<0.05$) and 24 h (versus 6 h, $P<0.01$). Adropin protein concentration in culture medium was positively correlated with the OD value at 12 h ($P=0.002$) and 24 h ($P=0.001$) after co-incubation.

CONCLUSIONS Atorvastatin promoted the expression of adropin in HUVEC and RASMC at a appropriate range of concentration, with the corresponding results that proliferation of HUVEC was promoted but RASMC proliferation was inhibited in the test concentration range of atorvastatin.